بسم الله الرحمن الرحيم

THE EFFECT OF DIFFERENT LEVELS OF CRUSHED *NIGELLA SATIVA* L. SEEDS ON GLUCOSE, INSULIN, CORTISOL AND BODY WEIGHT IN BROILERS

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DEDICATION

To my father
To my mother
To my husband
To my brother
To my sister and her daughters and son
With love and gratitude

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ABSTRACT

The aim of this study was to investigate the effect of feeding crushed *Nigella sativa* seeds on the plasma glucose levels, serum levels of insulin and cortisol hormones and live body weight of broilers chicks.

Hundred broiler chicks of one day old were used in this study. They were allotted randomized to three groups A, B and C.Group (A) (control group) received commercial ration, group (B) received commercial ration with 0.25% crushed *Nigella sativa* seeds, group (C) received commercial ration with 0.75% crushed *Nigella sativa* seeds.

The results showed that, at week (5) there was no significant decrease in blood glucose level in group B, however, there was a significant decrease ($P< 0.05$) in group C compared to the control group. At week (6) the groups B and C showed significant ($P< 0.01$) and ($P< 0.001$) decreased in blood glucose levels compared to the control group.

At week (5) there was no significant increase in serum insulin level in group B, while there was a significant increase ($P< 0.05$) in group C compared to the control group. At week (6), the groups B and C showed significantly ($P< 0.01$) and ($P< 0.001$) higher levels of insulin compared to the control group.

The levels of serum cortisol hormone were nonsignificantly different in the treated groups compared to the control group.

The result showed that live weight gain in the treated groups was not affected by dietary treatment compared to the control group.

خلاصة الأطروحة

هدفت هذه الدراسة إلي معرفة اثر التغذية بمسحوق بذور الكمون الأسود على مستوى الجلوكوز وهرموني الأنسولين و الكورتيزول في دم الأفراخ الاحمه إضافة إلي تأثيره على وزن الجسم الحي.

احتوت عينة الدراسة على مائة كتكوت عمر يوم واحد وقسمت العينة عشوائياً إلي ثلاثة مجموعات متساوية أ، ب وج ، حيث كانت المجموعة (أ) هي عينة التحكم أعطيت العلف التجاري للدجاج اللاحم خالية من الكمون الأسود، بينما أعطيت المجموعة (ب) بالإضافة للعلف التجاري مسحوق بذور الكمون الأسود بنسبة 25ر0% بينما أعطيت المجموعة (ج) بالإضافة للعلف التجاري مسحوق بذور الكمون الأسود بنسبة 75ر0%.

أوضحت النتائج في الأسبوع الخامس انخفاضاً معنوياً في جلكوز الدم في المجموعة (ج)، بينما لم يكن هنالك انخفاضاً معنوياً في المجموعة (ب) بالمقارنة مع مجموعة التحكم. أما في الأسبوع السادس كانت الزيادة معنوية وكبيرة في المجموعتين (ب) و(ج) على التوالي بالمقارنة مع مجموعة التحكم.

من ناحية أخري كانت هناك زيادة معنوية في تركيز الأنسولين في المجموعة (ج) في الأسبوع الخامس، بينما لم تتأثر المجموعة (ب)، أما في الأسبوع السادس كانت الزيادة معنوية وكبيرة في المجموعتين (ب) و(ج) على التوالي بالمقارنة مع مجموعة التحكم.
لم يكن هنالك تأثير معنوي على مستوي الكورتيزول في الدم في المجموعات المعالجة بالمقارنة مع مجموعة التحكم.
أيضاً لم يكن هنالك تأثير معنوي على وزن الجسم الحي في المجموعات المعالجة بالمقارنة مع مجموعة التحكم.

INTRODUCTION

Poultry is a good source of high quality protein in form of meat and eggs. So it is among the most efficient biological machines for reproduction and production that make it cheap for the consumer. However, feed accounts about 75% of the cost of poultry production (Shapiro, 2001).

Poultry industry in Sudan developed throughout 1970s, and since its development has been faced with many problems such as high cost of ration ingredients as sorghum, sesame and groundnut. This high cost is due to interaction of human and other animal requirements, moreover, the scarcity and high price of animal protein source. (Farah, 2001).

Recent research tended towards using non-conventional feed resources as additives in order to maximize the production and reduce the cost (Sideeg, 2000).

Nigella sativa which is commonly known as Habat El Baraka or El Haba Elsoda is an annual herb with aromatic odor (Maharan, 1967). This seed, as herbal medicament is traditionally taken in the morning to stimulate the appetite. The seeds are also used as a remedy for many diseases and general anti-poisons therapy (Bolous, 1993). *Nigella sativa* is a good source of essential amino acids such as lysine, methionine and minerals such as calcium, zinc and magnesium. Moreover *Nigella sativa* is of stimulant, aromatic, digestive, carminative, galactogogue, resolvent stomachic and tonic properties (Al-Jassir, 1992). In addition *Nigella sativa* seeds enhance immunity (El-Hag, 1998). Furthermore, it is a source of flavoring and

preservative agent, so it is used as condiment in bread, cakes and cheese (El Sayed *et al.*, 1997). The seeds were known to reduce blood glucose level (Kamal, 2006) and blood lipids (Farah, 2001).

The objectives of this study was to investigate the effect of feeding two levels of crushed *Nigella sativa* seeds on the plasma glucose, serum insulin, serum cortisol and body weight of broilers chicks. Aiming to explore the effect of feeding black cumin on these parameters which are key elements in carbohydrates metabolism and also used as indicators of stress conditions .

CHAPTER ONE

LITERATURE REVIEW

1.1 *Nigella sativa*

For thousands of years, human around the world have recognized the tremendous healing properties of legendary herb *Nigella sativa.* The oil from the black seeds (*Nigella sativa*) is known for thousands of years, human have regarded it as "Mircle Cure" (Nagi *et al.,* 1999).

1.1.1 Botanical back ground of the plant

Is an annual flowering plant, native to southwest Asia (Fig.1). *Nigella sativa* belongs to the order Ranunculaceae, 8 – 12 inches high, with finely divided, linear (but not thread-like) leaves. The flowers are delicate and usually coloured pale blue and white with 5 – 10 petals. Easily raised from the seed but dose not tolerate transplanting (Kypal, 1980 and Redgrove, 1993). The seeds are small about 2 – 3 mm, long more or less wedge-shaped and are intense dull black colour (Fig.2). They are markedly angular and their surface appears some what wrinkled. The fruit is a large and inflated capsule composed of 3 – 7 united follicles, each containing numerous seeds. The seed is used as a spice (Redgrove, 1993). The plant grows on the Mediterranean Coasts, in Egypt and other parts of the world including Saudi Arabia, Africa and parts of Asia. In Sudan the plant occurs naturally in Northern Sudan and Darfur State especially in Melliet and Jabel Marra (Andrews, 1990). The plant is successfully grown under low temperature and high humidity climates, so it is known as winter crop in Northern Africa and the Mediterranean and cultivated in October and November (Gulb, 1980).

Fig (1): *Nigella sativa* plant

Fig (2): *Nigella sativa* seeds

1.1.2 Chemical composition of the *Nigella sativa*

Nigella sativa seeds are rich in nutrients, organic compounds and minerals. The seed content of these compounds was investigated by Babayan *et al.* (1978). Compounds obtained from this seed are 35% fat, 0.8% volatile oil, 0.6% ash, 21% protein, and the rest being total of carbohydrates. Al Jassir (1992) showed that the seed component of amino acids were, arginine, methionine, lysine, glycine, leucine and glutamic acid. Also the fatty acids in *Nigella sativa* seed were, linoleic acid, oleic acid and palmatic asid. The component Nigellin resembles to turpentine, its soluble in water as well as in alcohol, though not in ether.

Abdel Majeed and Hassan (1999) showed the seed content of minerals (part per million) Table (1).

Al Jassir (1992) showed that the active ingredients in *Nigella sativa* seed are: thymoquinone (an alkaloid), nigellone (a carbonyl, polymer of thymoquinone and fixed oils). In the essential oil thymoquinone was identified as the main component beside β-cymene, α-pinene, dithymquinone and thymohydro-quinone.

1.1.3 The uses of *Nigella sativa*

Nigella sativa has been used for centuries, both as a herb and pressed into oil, by people in Asia, Middle East and Africa for medicinal purposes (Hatim, 1991).

Ibn Sina (980 – 1037 A.D), most famous for his volumes called ***The Canon of Medicine*** regarded by many as the most famous book in the history of medicine, refers to black cumin as the seed that stimulates the body's energy and helps recovery from fatigue and several

therapeutic effect on digestive disorders, gynecological diseases and respiratory system have been ascribed to the seeds of *Nigella sativa*.

It was used for different purposes in many countrie. Gutb (1980) mentioned that *Nigella sativa* seeds used for seasoning and flavoring of food. In the east generally they are used as a codiment to food and in Greece, Turkey and Egypt they are frequently strewed over the surface of bread and cakes in the same manner as sesame.

It is an excellent natural medicine used for millenniums treat a variety of conditions related to respiratory health, skin, stomach and intestinal disorders, kidney, liver function, circulatory and immune system support and to maintain and improve overall health (Abu Zeid, 1986).

It acts as a stimulant, aromatic, carminative, digestive, diuretic, emmenagogue, excitant, galactatagogue, purgative, resolvent, stomachic, sudorific, tonic, and vermifuge (Hatim, 1991).

Other research indicates that black seed contains the ability to significantly boost the human immune system, if taken overtime (El-Hag *et al.*, 1995).

Great research has been done on *Nigella sativa* in regards to it is anti-cancer properties, especially breast cancer with promising results, one of the largest experimental studies so far proved that *Nigella sativa* oil had numerous success in tumour therapy without side effects of common chemotherapy (Badary and Gamal Eldin, 2001).

Table (1): Mineral composition of *Nigella sativa*.

Mineral	Part per million
Ca	175.00
Mg	122.00
K	125.00
Fe	24.10
Mn	1.84
Zn	2.00
Na	70.00
Co	0.17
Cu	1.63

According to Abdel Majeed and Hassan (1999).

Bamosa *et al* (1997) found that Nigella sativa increased the growth rate of bone marrow cells. It stimulated immune cells and raised the interferon productions which protect cells from the cell destroying effect of viruses.

The oil of *Nigella sativa* is so beneficial due to its content of over a hundred components such as aromatic oils, trace elements, vitamins and enzymes. It contains 58% of essential fatty acids including omega 6 and omega 3. These are necessary for the forming of the prostaglandin E_1 which balances and strengthens the immune system giving it the power to prevent infection and allergies and control chronic illnesses (Houghton *et al.*, 1995).

Black seed oil contains about 0.5 – 1.5% volatile oils including nigellone and thymoquinone, which are responsible for its anti-histamine, anti-oxidant, anti-infective and broncho-dilating effect (El alfy *et al.*, 1975).

The healing secrets of black seed oil have been found to provide medicine properties ranging from immunostimulant, anti-bacterial, anti-fungal, anti-ulcerative, anti inflammatory, anti-oxidant, anti-tumourous, anti-pyretic, hypoglycaemic, immuno-modulatory, anti-hypertensive, antidepressant, anti-spasmic respiratory system rebuilder, hepato-protective, anti-parasitic and bronchodilator. As oil it is pass through the lymphatic consequently purifying and unblocking the lymphatic system (Musa, 1997).

For the upper respiratory conditions, at least a few of its constituents have shown an antihistamine – like action, which explains, its folk use as a glalactagogue. In large quantities, however, the seeds have also been used for the induction of abortion (El-Tahir *et al.*, 1993).

Externally the seed can be ground to a powder, mixed with a little flour as a binder and applied directly to abscesses, on the forehead for headache, nasal ulcers, orchitis and rheumatism.

1.1.4 *Nigella sativa* in poultry production

Best egg production and feed conversion ratio were shown by birds fed 0.5% and 1%. *Nigella sativa* and *Artimesia herba* during the treatment phase (Bakheit, 1998).

Sideeg (2000) found that supplementation with 0.25% *Nigella sativa* decreased abdominal fat of broiler chicks, and also the dietary level of 0.25% *Nigella sativa* lowered serum cholesterol.

Hamed (2003) showed that black cumin seed when given to laying hens diet at a dose rate of 1 – 3% for 3 months, resulted in significant reduction in the total lipids, total cholesterol, phospholipids and triglycerides compared to the control group. Also Hama, (2002) reported that feeding of *Nigella sativa* seeds to laying hens showed low level of total cholesterol in egg yolk when compared to the control groups.

The study by Ibrahim (2006) showed a significant decrease in the concentration of total cholesterol, triacylglycerols in the blood and liver. Also chicks received 15% and 20% of *Nigella sativa* added to the diet showed a decrease in serum total cholesterol level (Farah, 2001).

1.1.5 Effect of *Nigella sativa* on blood glucose and serum insulin level

It has been showed that ether petroleum extract of *Nigella sativa* (15 ml/kg Bwt) exerted an insulin-sensitizing action to rats, when injected subcutaneously daily (Phuong *et al*., 2001).

Also oral administration of water extract of *Nigella sativa* (30 ml/kg Bwt) significantly decreased serum glucose of diabetic rats from 19.8 mmol/l to 9.7 mmol/l and increased serum insulin from 0.5 mIU/l to 0.7 mIU/l. The results suggested the beneficial role of *Nigella sativa* as hypoglycemic agent and its protective effect against pancreatic β-cell damage, by decreasing oxidative stress and preserving pancreatic β-cell integrity and also suggest that the anti-diabetic effect of *Nigella sativa* may be attributed to increased glucose metabolism (Kamal, 2006).

Other studies by Meral *et al.* (2003) proved that, when used alloxan induced diabetic rabbits (oral doses of 20 mg/kg Bwt), there was a decrease in the blood glucose concentration.

A significant increase in serum insulin levels were observed after treatment of *Nigella sativa* with significant decrease in blood glucose level (Farah, 2002), the data showed that the hypoglycemic effect of *Nigella sativa* occur from stimulatory effect on β-cell function with consequent increase in serum insulin level.

Nigella sativa oil was reported to reduce blood glucose concentration and significantly increase serum insulin levels, when given orally to diabetic rats at a dose of 15 mg/kg Bwt (El Dakhakhny *et al*., 2002). Oral administration of ethanol extract of *Nigella sativa* seeds (300 mg/kg Bwt/day) to diabetic rats for 30 days significantly reduced the elevated levels of blood glucose (Mkaleem *et al.*, 2006). The result confirmed the antidiabetic activity of the extract of *Nigella sativa* seeds and suggested that because of its antioxidant effects its administration may be useful in controlling the diabetic complication in experimental diabetic rats.

Halit *et al,* (2002) proved that when *Nigella sativa* extract was received as intraperitoneal (IP) injection of (2 ml/kg Bwt) until the end of the study, it was found that *Nigella sativa* treatment increased the lowered insulin levels, and decreased the elevated glucose concentration of treated rats.

The mechanisms of the hypoglycemic effect of *Nigella sativa* was studied by Farah *et al*. (2004). They used alloxan diabetic hamsters and treated with *Nigella sativa* at a dose of 400 mg/kg Bwt, they found that *Nigella sativa* oil reduced blood glucose from 391 mg/dl before treatment to 325, 246, 208 and 179 mg/dl after the first, second, third and fourth weeks of treatment respectively. The studies suggested that hepatic glucose production from gluconeogenic precursors was significantly lower in treated hamsters, hence the hypoglycemic effect of *Nigella sativa* was suggested to be due to at least in part, a decrease in hepatic gluconeogensis and from a stimulatory effect on beta cell function with consequent increase in serum insulin level. Also they studied insulinotropic properties of *Nigella sativa* oil, significant increases in serum insulin levels were observed after treatment with *Nigella sativa*.

1.1.6 Effect of *Nigella sativa* on serum cortisol

Effect of *Nigella sativa* on cortisol level was studied by Kalus *et al.* (2003). In this study 152 patients with allergic disease were treated with *Nigella sativa* oil, given capsules at a dose of 40 to 30 mg/kg Bwt/day ,they found that cortisol levels remained unchanged.

Kamal (2006) used oral water extract of *Nigella sativa* in rats in a dose of 30 ml *Nigella sativa* extract /kg Bwt. The result showed a decrease in cortisol level.

1.1.7 Effect of *Nigella sativa* on body weight

Body weight is an important indicator of general health. *Nigella sativa* were fed orally to 7-old broiler chicks at 20 and 100 g/kg of the diet for 7 weeks. *Nigella sativa* diets did not adversely affect growth (Alhomidan *et al.,* 2002). Another three studies with regard to the influence of herbs (*Nigella sativa*) on growth were carried out with broilers over a period of 35 days, *Nigella sativa* supplement (10/20 g/kgBwt) orally. At the end of the period of the study, broiler body weight did not show any differences between the control animals and the groups fed *Nigella sativa* (Brake, 2004).

1.2 Insulin Hormone

Insulin is produced by the beta cells of the Islets of langerhans in the endocrine pancreas. The Islets contain glucagon, secreted by A-cells, and F cells or D cells which secrete pancreatic polypeptide. β-cells compose 60 – 80% of the Islets. Insulin consists of two polypeptide chains, both chains derived from a single prohormone (proinsulin). Proinsulin packaging into secretory vesicles. Secretory vesicles (proinsulin) undergoes proteolysis by many enzymes, this yields insulin Brain *et al*. (2001). Insulin has a diversity of actions on a range of tissues, the chief of which are liver, muscle and adipose tissues. Two broad categories of types of action may be recognized. It acts as a growth factor to promote cellular growth and differentiation and it plays a central role in the regulation of intermediary metabolism Brain *et al*. (2001).These two types of effects are mediated by the binding of insulin to its receptor which is to be found in the plasma membranes of all responsive tissues. Insulin act directly on muscle and adipose to increase their ability to take up glucose, thereby reduce the circulating

concentration of blood glucose. Insulin also promotes the storage of glycogen in muscle and in the liver, and in the later tissue it inhibits the conversion of glycogen into glucose and the process of gluconeogenesis. The action of insulin on lipid metabolism by inhibiting the release of fatty acids and glycerol from adipose tissue and also the liver, in the latter tissue the hormone promotes the synthesis of fatty acids and hence its export in the form of the triacylglycerols, also the hormone converts glucose to glycerol-3-phosphate and the conversion of this intermediate into triacylglycerols. Inhibition of lipolysis arises because insulin acts to reduce the activity of hormone-sensitive lipase which catalyses the hydrolysis of triacylglycerol. Insulin promotes the uptake of amino acids into muscle (also liver) (and hence protein synthesis) and it act to inhibit proteolysis. Although insulin is also antigluconeogenic in birds is not antilipolytic Brain *et al*. (2001).

The brain is particularly sensitive to glucose deficiency and nervous system dysfunction is seen. Initially confusion nervousness, trembling, hyper excitability may be seen. The body itself combats hypolycaemia through the release of insulin antagonistic hormones, catecholamines, glucagons, glucocorti-coids, and growth hormone.

Insulin deficiency is characterized by increased catabolism. The breakdown of glycogen and the decreased utilization of glucose in peripheral tissues lead to hyperglycemia. There is increased protein breakdown, and the increased released of amino acids fuels gluconcogenesis. Finally lipolysis leads to an increased fatty acid which transferred to the liver resulting in fatty liver and ketoacidosis Brain *et al*. (2001).

1.3 Cortisol Hormone

For many years cortisol has been referred to as a 'stress hormone' which is unfortunate because there are many grounds for thinking of it as just the opposite, an anti stress hormone. Cortisol produced from the adrenal cortex, which constitutes approximately 80 – 90 % of the mass of adrenal gland. The parenchymal cell of the cortex also called corticocytes, are the source of the various steroid hormones produced by the gland. The hormones produced are represented in each of the three major groups of steroid hormones: glucocorticoids, mineralocortioid and androgens. The most important glucocorticoids is the cortisol (Brain *et al*., 2001), cortisol synthesis begins with cholesterol. The levels are regulated by negative feed back system that depends upon interactions between a functional hypothalamic – pituitary – adrenal axis and the associated hormones. The most important components of this feedback system are corticotrophin releasing hormone CRH, adrenocorticotrophic hormone ACTH and cortisol, these are affected by the central nervous system or by other hormonal factor. In animals that are active during daylight the levels may be several times greater than during the last hours of daylight. All forms of stress, whether due to physical, chemical, thermal, microbial, or other factors, stimulate the hypothalamus to secrete corticotrophin releasing hormone CRH. Stress induced secretion of CRH up to 20-fold.

The more important effects of glucocorticoids are those related to the metabolism of energy substrates. Glucocorticoids protect the body from hypoglycemia during the post absorptive phase of digestion and during period of stress. In this regard it’s promoting the output of

hepatic glucose by stimulating glycogenolysis, enhance the effect of glucagon epinephrine on glucose availability and inhibit the effect of insulin (Roland *et al.,* 1998). Glucocorticoids stimulate the catabolism of protein, this lead to a negative nitrogen balance and increase in the urinary urea. Simultaneously inhibiting cellular uptake of amino acid, lead to increase amino acid in the circulation, and increase proteolysis in all tissues except the liver (uptake of amino acid and protein synthesis). Glucocorticoids are weak lipolytic agents that mobilize fatty acid from adipocytes and inhibit the uptake of fatty acids.

Cortisol antagonizes the effect of insulin on muscle, adipose tissues and liver and enhances the effect of glucagons and epinephrine.

Glucocorticoids cause wasting and atrophy of muscle tissues due to their catabolic effect. Glucocorticoids required for normal renal function and water metabolism, cortisol have some inherent mineralo-corticoids activity, due to its ability to binding to the mineralocorticoids receptors. Glucocorticoids most widely used as therapeutic agents due to their anti-inflammatory and immuno-suppressive properties. Glucocorticoids powerfully inhibit immune process including: secretion of cytokines produced by macrophages, helper T-cell, proliferation of immune cell, synthesis of antibody and cell mediated and humeral immune response. Hyper secretion of cortisol may result from tumours of adrenal gland, or tumors of the pituitary gland (rarely of the hypothalamus).

Massive release of glucocorticoids as result defense mechanism of animals is depressed and the animal are more susceptible to infection. Also polyuria and polydipsia are also clinical signs associated with hyper -secretion of glucocorticoids in animals. (Cheekes, 2005).

Hypo-secretion may be due to destruction of the adrenal gland leading to deficient production of cortisol lead to hypotension and hypoglycaemia. Hyposecretion of glucocorticoids impairs the ability of the kidneys to excrete a water load (Cheekes, 2005).

1.4 Blood Glucose

Glucose is a fuel substance used by most tissues under normal circumstances. Glucose is the "easiest" substrate for cells to utilize to release needed energy. In times of need, glucose is readily synthesized from non-carbohydrate sources (Hazelwood, 1986a). Glucose is the only substrate employed by certain tissues for energy purposes, although these tissues under stress frequently switch to other substrate for example the brain tissue. Effective homeostatic regulation of glucose metabolism invariably adjusts both protein and lipid metabolism normally, also the later two "depend" on normal metabolic disposition and/or production of glucose. Certain tissues (neural, retina and adrenal medulla) require glucose as it is their only substrate for maintaining normal function.

The healthy brain will require about 69 mg/hour of glucose. Because the demand for fuel is constant and our ability to deliver it is intermittent (due to largely tour meal eating habits), we must relay on a highly integrated series of regulatory mechanisms to sustain normal tissues cell activity. Glucoregulation therefore, is essential for the foregoing reasons in all animals and birds (Hazelwood, 1986b).

1.5 Body weight gain in broiler chicks

Genetic selection for growth over last 30 years has resulted in an average weight gain increase of 450 g/year (Lindemann *et al*., 1995).

Associated with this are an increase in fatness and loss of reproductive ability. Modern broilers contain 150 to 200 grams fat /kg Bwt. Fatness in poultry has three major attributes: depresses feed efficiency, some adipose tissues are of little economic value and consumption of saturated fat is associated with increased incidence of cardiovascular risks in humans. Therefore increase fat content in the chicken meat is undesirable both economically and socially (Reeds, 1987). Fats are required for normal growth and development, the essential fatty acids can not be synthesized by birds and therefore must be supplied in the feed, they are required for local mediators of metabolism, these polyunsaturated fatty acids have been reported to promote cell proliferation and reduce apoptosis rate (Tang *et al*., 1997).The growth of the broilers chicks depend on, diets supplement and environmental factors (Reilly *et al*., 1991).Temperature, air quality, humidity and light are critical factors to provide a comfortable, healthy environment for growing birds. Failure to provide the adequate environment during the brooding period will reduce profitability, resulting in reduced growth and development.

1.5.1 Temperature

Maintaining the correct temperature is crucial in chick brooding, especially during the first seven to ten days of the chick's life. Young chick is dependent on environmental temperature to maintain optimal body temperature. If the room temperature decreases, the chick body temperature will decrease likewise. If the room temperature increases, the chick body temperature will increase. Chilling or overheating during this crucial period can result in poor growth. The body temperature of a day-old chick is approximately 103 ^{o}F day (39^{o}C),

but by about five days of age body temperature is 106^{o}F (41.1^{o}C) the same as the adult. Extreme temperature (high or low) often results in chick mortality. Research has shown chicks that are subjected to cold temperature have impaired immune and digestive system. As a result, cold stress have reduced growth and increased susceptibility to disease. Not only do chicks exposed to low brooding temperature have reduced growth rate, but they will consume more feed to keep themselves warm, reducing feed efficiency and increased feed cost (Czarick *et al*., 2001).

1.5.2 Humidity

The ability of air to hold moisture depends upon its temperature, warm air can hold more moisture than cold air. The term humidity refers to the percent of water saturation of air at any temperature.

It is recommended that a low level of humidity to be maintain during bird brooding. Increased humidity improves environmental conditions for microbial growth in the litter. As the microbial population increases, more ammonia generated from nitrogen sources found in bird fecal material. Ammonia is a gas that has negative impact on bird's health and performance. Research shows that increased ammonia impairs the immune system, and increases respiratory diseases in bird. High ammonia levels during brooding reduces growth rate, which is not gained back during the remainder of the growth period. Ammonia production can be reduced through the control of relative humidity which in turn regulated by ventilation. A relative humidity level of 50% to 70% is recommended to minimize ammonia production and dust (Carlile, 1984).

1.5.3 Ventilation

Ventilation is needed to regulate temperature and remove carbon dioxide, ammonia, other gases, moisture, dust and odors. The flow pattern within the building is very important. Mixing of the incoming outside air and the inside air prevents the cooler air from settling near the liter and chilling the birds. This not only helps maintain bird's body and floor temperature but also helps remove moisture from the litter (Czarick, 2001).

1.5.4 Lighting during breeding

Light is an important factor during breeding that cannot be ignored. Chick activity is greater in bright light intensity than low light intensity. Light should be at the brightest intensity to encourage chick activity thus assisting them to locate feed and water. The light system design should allow light intensity and duration to be modified as the birds age (Fairchild *et al*., 2003).

CHAPTER TWO
MATERIALS AND METHODS

The experiment was carried out in the premises of the faculty of Animal Production, University of Khartoum during the period between December – January (2005 – 2006).The minimum and maximum temperature were 15ºC and 35ºC ,and under humidity of (21% to 35 %). The experiment lasted for six weeks.

2.1 Experimental house

The house used was with a concrete floor and open mesh sides set over a half meter brick barrier. The eastern and western sides were covered with jute sacks to prevent conventional heat effects and to control the direct sunrays. Feed and water were provided *ad libitum*. The house was cleaned before use, by fire, washed and disinfected using malathion. Each room floor was covered with enough wood shaving with allocation of two tubular feed troughs and two round fountain drinkers.

2.2 Experimental birds

One hundred one day old unsexed commercial broiler chicks (ROSS) were purchased from Arab Poultry Breeders Co. (Coral) Hatcheries, Khartoum and transferred to the Animal Production Unit.

Upon arrival chicks were divided into two groups of 50 chicks, each group were allocated at random to pens, they were fed the control diet *ad libitum* for one week.

They were offered water fortified with antibiotic for 3 days. Also vitamins and minerals premix offered for 5 days. After 7 days the hundred healthy chicks of approximately equal live weight were randomly allotted into three treatments A, B, C each with two replicate groups, approximately 16 chicks in each pen. The broiler chicks were vaccinated against new castle, at the second week of age and revaccinated at the third week of age, and also vaccinated against gumboro disease.

2.3 Management

Standard management practices were adopted for each group. The first 3 days of life feed was offered in semi-flat chick trough for easy access. These were replaced later by round feeders. Every day throughout the experimental period, the house interance was disinfected early in the morning using malathion. The house was cleaned every day. Daily chick waterers were washed and the wood shaving bedding was changed. Every week the live weight was recorded. The lighting cycle was 24 hours. The average room temperature and humidity throughout the experiment period were recorded.

2.4 Experimental diets

The experimental diets were purchased from Arab Poultry Breeders Co. (Coral) in Khartoum. All nutrient requirements of broiler ration were formulated in the ration. The ingredients of the basal ration shown in Table (3).

Nigella sativa seeds were purchased from the local market (Omdurman market).The chemical analysis of *Nigella sativa* was shown in Table (2). The chicks divided into three groups A, B and C.

Group (A): Received a basal diet, served as a control.

Group (B): Received a basal diet containing 0.25% crushed *Nigella sativa* seeds.

Group (C): Received a basal diet containing 0.75% crushed *Nigella sativa* seeds.

Table (2): Chemical composition of *Nigella sativa*

Constituents	%
Ether extract	35.58
Crude protein	28.35
Dry matter	24.31
Ash	4.23
Crude fiber	7.53

According to Babayan *et al*. (1978).

Table (3): Composition of the basal ration

Ingredients	%
Crude protein	23.00
Lysine	1.25
Methionine	0.41
Crude fiber	3.00
Crude lipid	4.20
Calcium	1.00
Total phosphorus	0.50

According to Arab Poultry Breeders Corporation (Coral).

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The basal ration was fed to the birds for an adaptation period of one week. Then they were immediately introduced to their subjective experimental ration. The experimental feeding continued for 5 weeks.

2.5 Sample collection

After the fifth week of the experiment, 5 samples were collected randomly from each replicate of chicks. Blood samples were taken from chick's veins after disinfection of the area using alcohol. The blood samples were transferred into plain tubes allowed to clot and centrifuged at 3000 rpm for 5 minutes, the separated serum was stored at -20^{o}C until used for hormone assay.

The blood samples for glucose determination were taken into fluoride oxalate containers and immediately centrifuged and plasma was decanted and stored at 4^{o}C for one day. The second samples were taken after six weeks of the experiment, and the levels of hormones and glucose concentration were determined.

2.6 Biochemical analysis

Blood samples collected from all groups were used to measure the levels of blood glucose concentration, insulin and cortisol hormones. The analysis of the hormones was done in the Sudan Atomic Energy Commission, Institute Of Radiobiology and Radioimmunoassay Laboratory. Analysis of glucose was done in Khartoum Educational Hospital.

2.6.1 Determination of plasma glucose level

Glucose estimation was performed by using glucose oxidase method, in the fluoride oxalate plasma samples,according to Thomas, (1998).

Principle

In the presence of glucose oxidase, glucose is oxidized to hydrogen peroxide and gluonate. Hydrogen peroxide reacts, in the presence of peroxidase with phenol and 4-aminophenazone to form a quinoneimine dye. The intensity of the pink colour formed is proportional to the glucose concentration.

Reagents:-

- Phosphate buffer, pH 7.5.
- Phenol.
- 4-aminoantipyrine.
- Glucose oxidase.
- Peroxidase.

Procedure

1. Prepare three test tube, blank tube, standard tube, sample tube.
2. Put in each tube 1000 µl of reagent.
3. Put 10 µl of sample in sample tube.
4. Put 10 µl from standard in standard tube.
5. Mix incubates for 10 min. at 37^{o}C or 20 min, at 20 – 25^{o}C.
6. Measure absorbance of sample, and standard within 60 min against reagent blank.

Calculation

$$\text{Glucose (mg/dl)} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times \text{conc. of standard (mg/dl)}$$

mg/dl X 0.055 = mmol/l

2.6.2 Determination of insulin level in the serum

Insulin measurement was done by using radioimmunoassay kit (RIA), which provides rapid and sensitive procedure for the quantitative measurement of insulin in serum according to method described by Beijing, (1995).

Principle of the procedure

The radioimmunoassay method in this test depends upon the competition between iodine-125 labeled insulin and insulin in serum sample, for the limited number of binding site on insulin specific antibody. After incubation for a fixed time and separation of bound from free. The tubes then counted in gamma counter the counts being inversely related to the amount of insulin present in sample.

Contents of the kit

1. 1 vials freeze-dried iodine-125 labeled insulin (red).
2. 6 vials of freeze-dried insulin standards.
3. 1 vial freeze-dried insulin antibody (blue).
4. 1 vial of buffer.
5. 1 bottle of 52 ml separating agent solution.

Procedure

1. The tubes arranged according to the scheme in Table below.
2. 100 µl of buffer added to insulin standard or unknown sample.
3. 100 µl insulin antibody solution added to all tubes except total and NSB tube.
4. 100 µl of iodine-125 labeled insulin solution to all tubes was added, incubate at 37^{o}C for 2 hours.

5. 500 µl of separating agent solution was added to all tubes except total count tube incubates and mixed for 15 min at 37°C.
6. All the tubes were centrifuged (except those for total counts) for 15 min.
7. The supernatant of all tubes was decanted.
8. The tubes were counted in a gamma counter.

	Total counts	Standard (ml µl)								Un-known sample
		NSB	0	5	10	20	40	80	160	
Buffer		20	100							
Standard				100	100	100	100	100	100	
Sample										100
Antibody			100	100	100	100	100	100	100	
^{125}I-insulin*	100			100	100	100	100	100	100	100
Separating reagent		500	500	500	500	500	500	500	500	500

* Mixed thoroughly and incubate at 37°C for 2 hrs.

Calculations

$$\frac{B\%}{B_0} = \frac{CPM\ of\quad Qc,\quad STD,\quad Sample}{s_0} \times 100\%$$

- B : Binding antibody.
- CPM : Count per minute.
- Qc : Quality control.

Plot in linear percent values of each standard on the vertical axis against insulin concentration in the horizontal axis.

2.6.3 Determination of Cortisol level in the serum

Cortisol analysis was conducted by (RIA) Radiommunoassay according the method described by Prasad, (1990).

Principle

This assay is based on the competition between cortisol in sample (unlabeled) and fixed quantity of (125 labeled cortisol (B) for a limited number of binding sites on cortisol specific antibody. The amount of labeled cortisol 125-I bound by the antibody will be inversely proportional to the concentration of unlabeled cortisol. By measuring the proportion of 125-I bound in the presence of reference standards containing various known amount of cortisol, the concentration of present in unknown sample can be interpolated.

Reagent

1. Tracer (labeled cortisol 125-I) (1 vial).
2. Standards (6 vials).
3. Antiserum (polyclonal anti-cortisol) IgG (1 vial).
4. Control serum (1 vial).
5. Magnetic immunosorbent (1 bottle).

Procedure

1. Label duplicate tubes for total counts (T), zero standards (standard 1= B_0) standard (S_{2-6}), control © and samples (S_x).
2. Vortex mix.
3. Incubate for 2 hours at room temperature.

Tubes	T	S_{1-6}	S_x	C
Standard		10		
Sample			10	
Control				10
Tracer	100	100	100	100
Antiserum		100	100	100

$S_x \equiv$ Sample

T ≡ Total count

$S_{1-6} \equiv$ Standards

C ≡ Control

4. Magnetic immunosorbent 500 500 500.
5. Vortex mix.
6. Incubate for 15 minutes at room temperature.
7. Place the tubes on the magnetic separator for 5 minutes or centrifuge for 15 minutes.
8. Count all tubes.

Calculation of result

$$\frac{B\%}{B_0} = \frac{CPM\ of\quad Qc,\quad STD,\quad Sample}{s_0} \times 100\%$$

- B: Binding antibody.
- CPM: Count per minute.
- Qc: Quality control.

2.7 Statistical analysis

The data were subjected to completely randomized design.Analysis of variance (ANOVA) and mean separation were conducted to test significant differences of groups according Steel and Torie (1980).

CHAPTER THREE
RESULTS

The present study was carried out to investigate the effect of feeding crushed *Nigella sativa* seeds on the levels, of plasma glucose, serum insulin, serum cortisol (as stress indicators) and body weight of broiler chicks.

3.1 The effect of dietary crushed *Nigella sativa* seeds on plasma glucose level (mg/dl) in broiler chicks

The result of plasma glucose levels of the groups fed 0.00%, 0.25% and 0.75% crushed *Nigella sativa* seeds are presented in Table (4) and Fig. (3).

The results taken after five weeks showed that, the group fed 0.25% crushed *Nigella sativa* seeds was not affected by dietary treatment compared to the control group, but there was a significant decrease ($P < 0.05$) in the group fed 0.75% crushed *Nigella sativa* seeds compared to the control group.

At week six there was a significant ($p<0.05$) decrease in plasma glucose in both groups treated with crushed *Nigella sativa* seeds (group B and C).

Table (4): The effect of dietary crushed *Nigella sativa* seeds in plasma glucose level (mg/dl) in broiler chicks.

Groups	Means ± SE	
	Week 5th	Week 6th
A	158 ± 1.75	249 ± 2.36
B	149 ± 2.10	224± 6.27
C	139 ± 3.40	130 ± 4.78

Group treatment

Group A: received a standard diet.

Group B: received a standard diet with 0.25% *Nigella sativa* seeds.

Group C: received a standard diet with 0.75% *Nigella sativa* seeds.

SE: Standard Error.

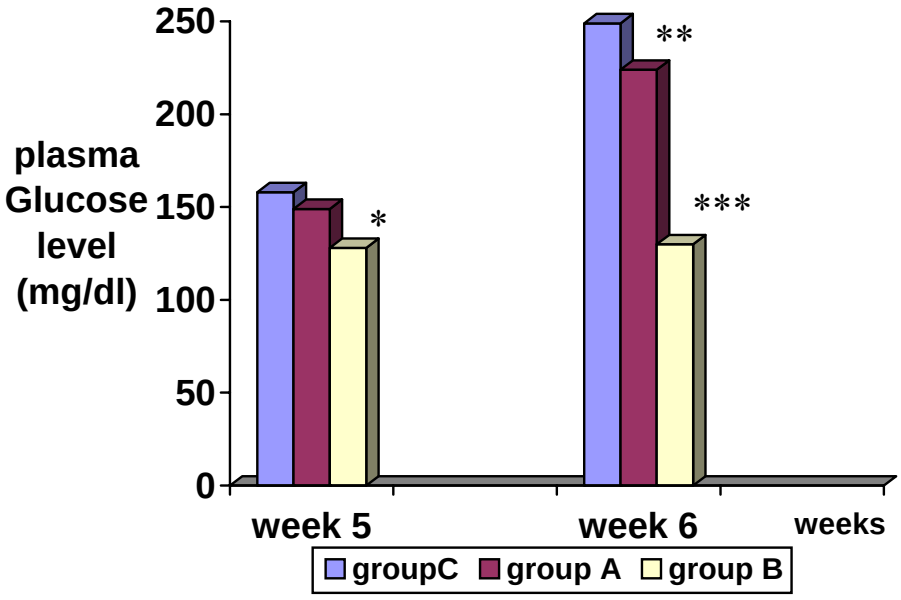
Means compared to control levels having

* = ($P< 0.05$).

** = ($P< 0.01$).

*** = ($P< 0.001$).

Fig (3): The effect of dietary crushed *Nigella sativa* seeds on plasma Glucose level (mg/dl) in broiler chicks



Group treatment

Group A: received a standard diet.

Group B: received a standard diet with 0.25% *Nigella sativa* seeds.

Group C: received a standard diet with 0.75% *Nigella sativa* seeds.

Means compared to control levels having

* = ($P < 0.05$).

** = ($P < 0.01$).

*** = ($P < 0.001$).

3.2 The effect of dietary crushed *Nigella sativa* seeds on serum insulin level (mIU/ml) in broiler chicks

The result of serum insulin levels of group fed 0.00%, 0.25% and 0.75% crushed *Nigella sativa* seeds are presented in Table (5) and Fig (4). In the fifth week the serum insulin levels were higher in both treated group, the group fed 0.25% crushed *Nigella sativa* seeds it was not significant increased compared to the control group, while there was a highly significant ($P < 0.01$) increase of insulin level in the group fed 0.75% crushed *Nigella sativa* seeds.

Also in the sixth week, the group fed 0.75% crushed *Nigella sativa* seeds, insulin level increase to a very high significantly ($P < 0.001$) value compared to the control group, while there was also highly significant ($P < 0.01$) increase in group fed 0.25% crushed *Nigella sativa* seeds.

3.3 The effect of dietary crushed *Nigella sativa* seeds on serum cortisol level (ng/ml) in broiler chicks

The result of serum cortisol levels of group fed 0.00%, 0.25% and 0.75% crushed *Nigella sativa* seeds are presented in Table (6) and Fig (5). The serum cortisol levels was not affected by dietary *Nigella sativa* treatment, and showed no significant difference in both group fed 0.25% and 0.75% crushed *Nigella sativa* seeds compared to the control group.

Table (5): The effect of dietary crushed *Nigella sativa* seeds on serum insulin level (mIU/ml) in broiler chicks.

Groups	Means ± SE	
	Week 5	Week 6
C	3.1 ± 1.49	4.0 ± 2.84
A	4.5± 1.0	7.8 ± 1.3**
B	9.1± 1.9**	10.7 ± 2.0***

Group treatment

Group A: received a standard diet.

Group B: received a standard diet with 0.25% *Nigella sativa* seeds.

Group C: received a standard diet with 0.75% *Nigella sativa* seeds.

SE: Standard Error.

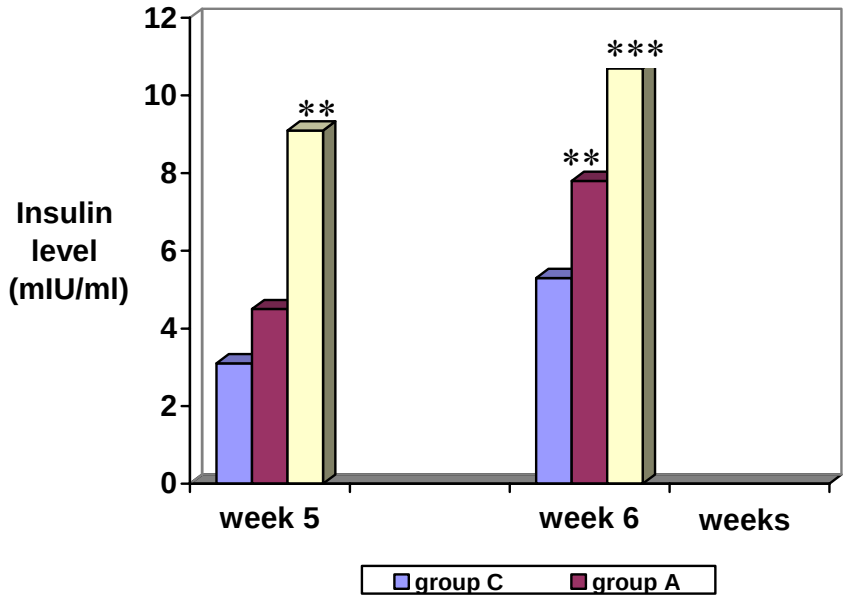
Means compared to control levels having

* = ($P< 0.05$).

** = ($P< 0.01$).

*** = ($P< 0.001$).

Fig (4): The effect of dietary crushed *Nigella sativa* seeds on serum insulin level (mIU/ml) in broiler chicks.



Group treatment

Group A: received a standard diet.

Group B: received a standard diet with 0.25% *Nigella sativa* seeds.

Group C: received a standard diet with 0.75% *Nigella sativa* seeds.

Means compared to control levels having

* = ($P< 0.05$).

** = ($P< 0.01$).

*** = ($P< 0.001$).

Table (6): The effect of dietary crushed *Nigella sativa* seeds on serum cortisol level (ng/ml) in broiler chicks

Groups	Means ± SE	
	Week 5	Week 6
C	4.0 ± 0.54	4.4 ± 0.53
A	4.3± 0.65	4.5 ± 0.45
B	4.0± 0.55	5.0 ± 0.49

Group treatment

Group A: received a standard diet.

Group B: received a standard diet with 0.25% *Nigella sativa* seeds.

Group C: received a standard diet with 0.75% *Nigella sativa* seeds.

SE: Standard Error.

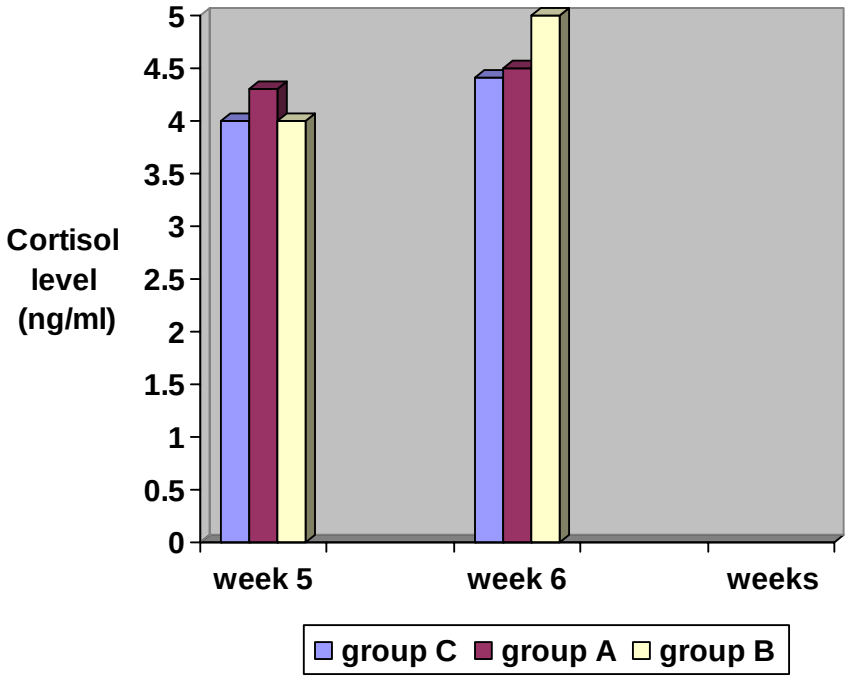
Means compared to control levels having

* = (P< 0.05).

** = (P< 0.01).

*** = (P< 0.001).

Fig (5): The effect of dietary crushed *Nigella sativa* seeds on serum cortisol level (ng/ml) in broiler chicks



Group treatment

Group A: received a standard diet.

Group B: received a standard diet with 0.25% *Nigella sativa* seeds.

Group C: received a standard diet with 0.75% *Nigella sativa* seeds.

Means compared to control levels having

* = ($P< 0.05$).

** = ($P< 0.01$).

*** = ($P< 0.001$).

3.4 The effect of dietary crushed *Nigella sativa* seeds on body weight gain of broiler chicks

The changes in body weight of broilers fed 0.00%, 0.25% and 0.75% crushed *Nigella sativa* seeds are presented in Table. (7and 8) and Fig. (6 and 7).

In Figure (7) all treated groups showed similar growth rate, however from 2nd to 3rd week of age birds fed 0.75 % crushed *Nigella sativa* seeds (group B) exhibited the highest body weight. Then in the 4 th week of age control group had the best body weight, and at 5 th week of age bird fed 0.25 % crushed *Nigella sativa* seeds (group A) had the best body weight, at the last week the control group and the group fed 0.25 % crushed *Nigella sativa* seeds had numerically the highest body weight.

After week of age the increase in body weight from the start weight fallowed the increase of the level of the *Nigella sativa* as 77%, 81% and 84% for the group fed 0.00%, 0.25% and 0.75% respectively Table (8).At week 3 the growth rate decreased in all groups and this was obvious in the control group, which presented 61% as increase only compared to 63% and 68% for the group fed 0.75% and 0.25% crushed *Nigella sativa* seeds respectively. The rate of growth increase again in the week 4 but still in the group fed 0.75% crushed *Nigella sativa* seeds showed the lowest rate of growth compared to the groups. Towards week 5 the growth rate obviously decreased again in all groups compared to the previous weeks. At week 6 the rate of growth increased again and the group fed 0.75% crushed *Nigella sativa* seeds showed the highest rate compared to the other groups.

Table (7): The effect of dietary *Nigella sativa* on body weight (g/bird/week) of broiler chicks.

Groups	Means ± SE			
	0.00 % *N.S*	0.25 % *N.S*	0.75% *N.S*	SE
1st week	175	160	173.8	6.6^{NS}
2nd week	310	290	320	4.3^{NS}
3rd week	500	490	520	18^{NS}
4th week	925	912.5	875	33^{NS}
5th week	1250	1275	1115	31^{NS}
6th week	2225	2225	2060	36^{NS}

N.S: Nigella sativa.

NS: non significant.

Table (8): The effect of dietary *Nigella sativa* on the increase body weight (percentage/week) of broiler chicks

weeks	Means ± SE			
	0.00 % *N.S*	0.25 % *N.S*	0.75 % *N.S*	SE
Week 2	77	81	84	4.3
Week 3	61	68	63	18
Week 4	85	86	68	33
Week 5	35	39.7	27	31
Week 6	78	75	85	36

N.S: *Nigella sativa.*

Fig. (6): The effect of dietary *Nigella sativa* on body weight gain (g/bird/week) of broiler chicks.

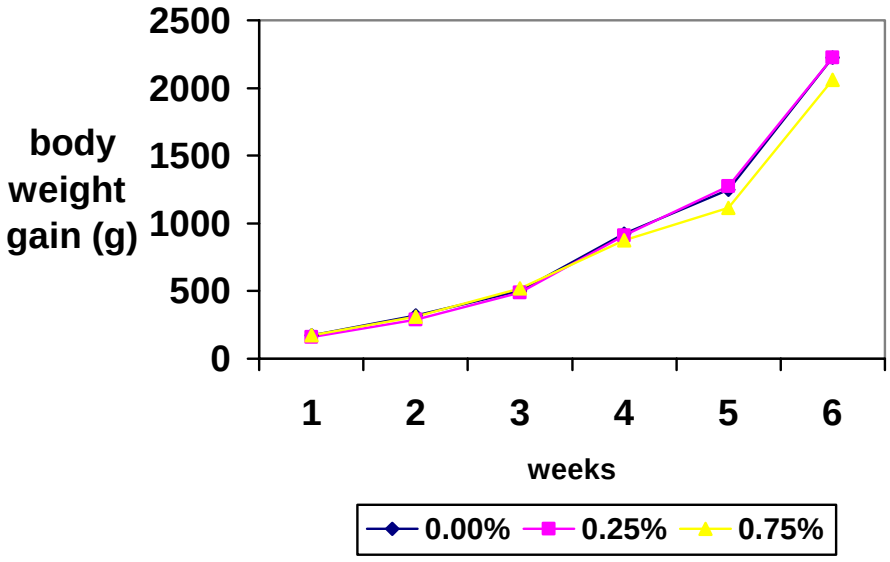
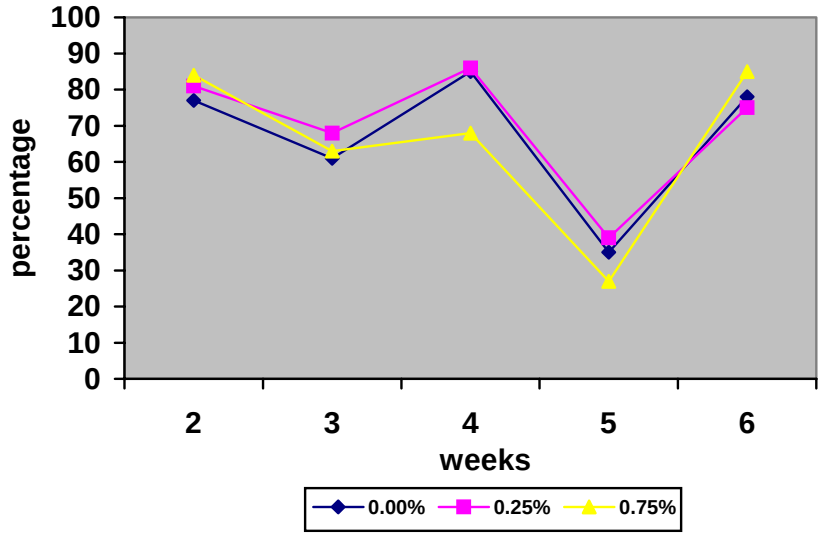


Fig. (7): The effect of dietary *Nigella sativa* on body weight (percentage/bird/week) of broiler chicks



CHAPTER FOUR
DISCUSSION

The present study was caried out to investigate the effect of crushed *Nigella sativa* seeds on plasma glucose, serum insulin,serum cortisol hormones and body weight of broiler chicks. This plant is known in traditional and folklore medicine for very long time to be used for treatment of diseases in man and animals. Several investigators have studied the effect of *Nigella sativa* on diabetes-mellitus, and proved that it reduces blood glucose levels in diabetic groups, and also elevated serum insulin hormone. Others reported the effect of this plant on cortisol hormone and body weight of broiler chicks (EL Kadi and Kandial, 1997; Ali and Gerald, 2003).

4.1 The effect of feeding crushed *Nigella sativa* seeds on serum total glucose in broilers

Blood glucose levels were decreased significantly in the groups treated with crushed *Nigella sativa* seeds, compared to the control group. However the group fed higher concentration (0.75%) of crushed *Nigella sativa* seeds showed lower level than the group fed (25%) of crushed *Nigella sativa* seeds. These results were in line with the reports from previous literature which suggested that administration of ether petroleum extract and water extract of *Nigella sativa* seeds to animals (rat, rabbits and chicks) is effective in decreasing blood glucose levels (Eskander *et al*, 1998; Halit *et al*, 2002 and Meral *et al*, 2003).

Mkaleem *et al,* (2006) have also demonstrated the antidiabetic effect of *Nigella sativa* seeds when used as ethanol extract. The result confirmed the antidiabetic activity of *Nigella sativa* seeds and suggested that because of it's antioxidant effect, it may be used in controlling the diabetic complications.The present study proved that supplementation of crushed whole seeds also reduced blood glucose significantly in broiler chicks.

The mechanism of glucose reduction was reported by Kamal, (2006), he suggested that *Nigella sativa* seeds induce preserving pancreatic β-cell integrity, this may be attributed to increased glucose metabolism, by increasing the serum insulin. Finding in the present study support this idia by reporting significant increase in insulin levels in the treated grruop. But Fararh *et al.* (2004) suggested that the reduction of blood glucose from 391 before treatment to 179 mg/dl in hamsters after treatment is due to significantly lower in hepatic glucose production from gluconeogenic precursors, hence the hypoglycaemic effect is due to a decrease in hepatic gluconeogensis.

4.2 The effect of feeding crushed *Nigella sativa* seeds on serum insulin in broilers

In the present study crushed *Nigella sativa* seeds caused significant increase in serum insulin levels in the groups treated with 0.75% and 0.25% crushed *Nigella sativa* seeds. These results agree with Phuong *et al,* (2001),as he reported that petroleum extract of *Nigella sativa* seeds exert insulin-sensitizing action in rats, by mechanism of enhancing the activity of intracellular signal transduction pathway of the hormone receptor, hence increase the insulin levels.

The increase in serum insulin level was also observed by Fararh, (2002), who suggested that the increase in serum insulin levels resulted from stimulatory effect on β-cell function.Which indicated that *Nigella sativa* has insulinotropic properties.

Halit *et al,* (2002) reported similar result, that *Nigella sativa* treatment increased the lowered insulin levels in rats, and it was confirmed by Rchid *et al,* (2005) he reported that using of different fractions of the seeds caused significant increase in insulin production from β-cell of rats.

4.3 The effect of feeding crushed *Nigella sativa* seeds on serum cortisol in broilers

In the present work serum cortisol levels were not affected by dietary crushed *Nigella sativa* seeds, and showed no significant difference in both groups fed 0.25% and 0.75% crushed *Nigella sativa* seeds, thus theses groups showed a control like levels. These findings agree with Kalus *et al,* (2003) who reported that, administration of *Nigella sativa* to patients with allergic disease, showed no significant effect on serum total cortisol, but this finding contradicted with that reported by Kamal *et al,* (2006) who reported that administration of water extract of *Nigella sativa* to rats exerted decreased in cortisol level, he reported that the plant mechanism of action is still largely unknown, due to lack of study data on it.

4.4 The effect of feeding crushed *Nigella sativa* seeds on broilers Body weight

In the present study all treated groups showed similar growth rate. However from 2nd to 3rd week of age bird fed 0.75% crushed *Nigella sativa* seeds exhibited maximum increase of body weight, then in the 4th week of age the control group has the best body weight Table (7). In the last week the control group and the bird fed 0.25% crushed *Nigella sativa* seeds showed numerically increase of body weight.

Table (8) showed increasing on body weight by percentage/week, at week 2 of age the increase in body weight followed the increase of the level of *Nigella sativa*, at week 5 the growth rate decrease dramatically in all groups, these decrease might be attributed to disease. Alhomidan *et al*, (2002), reported that, feeding 20 and 100 g/kg body weight of *Nigella sativa* seeds to 7 day old broiler chicks did not affect in growth. Also Brake, (2004) reported that administration of *Nigella sativa* seeds did not causes any differences between control animals and treated one. Another study done by Abdel Majeed and Hassan, (1999), they reported that dietary *Nigella sativa* had no affected on growth. But this finding contradicted with that reported by Al-Jassir, (1992) he demonstrated that, the hens received 0.25% *Nigella sativa* recorded highest body weight and he suggested that this may be due to the nutritive value of *Nigella sativa*.

CONCLUSIONS

It is concluded from this study that crushed *Nigella sativa* seeds, supplemented to the diets of broiler chicks, decreased significantly the levels of plasma glucose, and increased significantly the levels of insulin hormone, suggesting that this seeds could be used as a hypoglycemic agent to reduced blood glucose level, while the level of cortisol hormone was not affect by treatment.

Also live weight gain in treated group was not affected by dietary treatment compared to the control group.

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